HEK-Blue™ hACE2 Cells

SEAP reporter HEK293 cells expressing human ACE2 gene

Catalog code: hkb-hace2

https://www.invivogen.com/hek-blue-hace2-cells

For research use only

Version 21F10-ED

PRODUCT INFORMATION

Contents and Storage

- **3-7 x 10^6 HEK-Blue™ hACE2 cells in a cryovial or shipping flask**

IMPORTANT: If cells provided in a cryovial are not frozen upon arrival, contact InvivoGen immediately.

- **1 ml of Zeocin™** (100 mg/ml), store at 4 °C or at -20 °C.*
- **1 ml of Puromycin** (10 mg/ml), store at 4 °C or at -20 °C.*
- **1 ml of Normocin™** (50 mg/ml): a formulation of three antibiotics active against mycoplasmas, bacteria and fungi. Store at -20°C.*

*The expiry date is specified on the product label.

- **1 ml of QB reagent and 1 ml of QB buffer** (sufficient to prepare 100 ml of **QUANTI-Blue™ Solution**, a SEAP detection reagent). QB reagent and QB buffer are stable for 1 year at -20 °C. QUANTI-Blue™ Solution is stable for 2 weeks at 4 °C and for 2 months at -20 °C.

Note: Data sheets for all components are available on our website.

Handling Frozen Cells Upon Arrival

Cells must be thawed immediately upon receipt and grown according to handling procedures (as described on the next page) to ensure the best cell viability and proper assay performance.

Note: **Avoid freezing cells upon receipt** *as it may result in irreversible damage to the cell line.*

Disclaimer: We cannot guarantee cell viability if the cells are not thawed immediately upon receipt and grown according to handling procedures.

IMPORTANT: For cells that arrive in a shipping flask please refer to the enclosed 'cell recovery procedure'.

Cell Line Stability

Cells will undergo genotypic changes over time resulting in reduced responsiveness in normal cell culture conditions. Genetic instability is a biological phenomenon that occurs in all stably transfected cells. Therefore, it is critical to prepare an adequate number of frozen stocks at early passages. HEK-Blue™ hACE2 cells should not be passaged more than 20 times to remain fully functional.

Quality Control

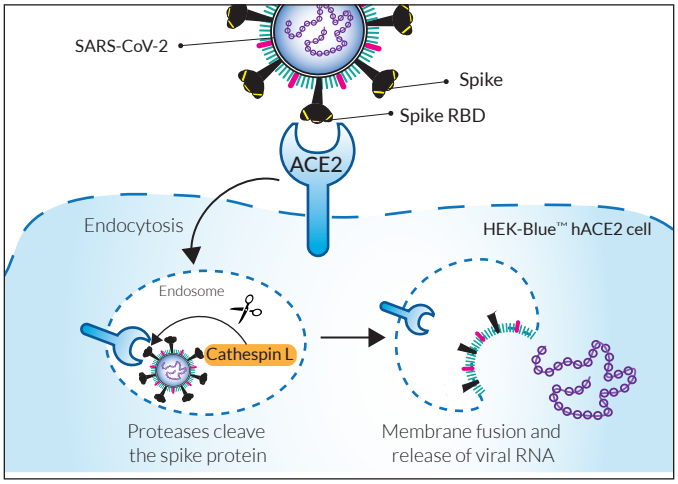
- The overexpression of the human *ACE2* (hACE2) gene has been verified by RT-qPCR, FACS staining, and functional assays.
- The stability for 20 passages following thawing has been verified.
- These cells are guaranteed mycoplasma-free.

CELL LINE DESCRIPTION

HEK-Blue™ hACE2 cells were generated from HEK-Blue™ Null1-v cells, which derive from the human embryonic kidney (HEK)-293 cell line. HEK-Blue™ hACE2 cells overexpress the human ACE2 (hACE2) gene. Thus, unlike their parental cell line, they are permissive to infection with pseudotyped lentiviruses expressing the SARS-CoV-2 Spike protein. Additionally, they express a secreted embryonic alkaline phosphatase (SEAP) under the control of an NF-κB inducible promoter comprised of an IFN-β minimal promoter fused to five NF-κB and AP-1 binding sites. Levels of SEAP in the supernatant can be easily determined with QUANTI-Blue™ Solution, a SEAP detection cell culture medium. HEK-Blue™ hACE2 cells are resistant to Puromycin and Zeocin™.

BACKGROUND

ACE2 (angiotensin I-converting enzyme-2) is a type I membrane protein that belongs to the angiotensin-converting enzyme family. It is established as host receptor for the Spike protein of SARS-CoV-2, the causative agent of COVID-19[1,2]. Specifically, SARS-CoV-2 gains entry to host cells through the binding of the Spike receptor-binding domain (RBD) to ACE2 at the cell surface[1,3]. Following this, the host protease, TMPRSS2, cleaves the S protein into two subunits (S1 and S2), mediating the fusion between the viral and host membranes[1,3]. Notably, TMPRSS2 is not needed for infection of HEK293 cells by SARS-CoV-2 spike-pseudotyped lentiviral particles[4].



1. Hoffmann M. *et al.* 2020. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 181:1-16. 2. Zhou P. *et al.*, 2020. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 579(7798):270-273 3. Walls A.C. *et al.* 2020. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell. 181(2):281- 292.e6. 7. Korber B. *et al.*, 2020. Tracking changes in SARS-CoV-2-Spike: evidence that D614G increases infectivity of the COVID-19 virus. Cell. DOI: 10.1016/j.cell.2020.06.043.

APPLICATIONS

HEK-Blue™ hACE2 cells are sensitive to infection by SARS-CoV-2 and/or spike-pseudotyped lentiviral particles. They are ideal for studying viral entry into host cells, as well as for screening small molecule inhibitors and neutralizing antibodies. In addition, these cells express an NF-κB-inducible SEAP reporter and therefore, can be used as 'acceptor' cells in combination with InvivoGen's 293-hMyD88 cells (donor cells) to study cell fusion (*for a detailed protocol see other side*).

Note: For more information visit https://www.invivogen.com/cell-fusion

USER RESTRICTIONS

These cells are distributed for research purposes only.

This product is covered by a Limited Use License. By use of this product the buyer agrees to the terms and conditions of all applicable Limited Use Label Licenses. For non-research use, such as screening, quality control or clinical development, contact info@invivogen.com.

TECHNICAL SUPPORT
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FAQ Any questions about our cell lines?
Visit our FAQ page.

SAFETY CONSIDERATIONS

Biosafety Level 2

HEK-Blue™ hACE2 cells were derived from HEK293 cells (transformed with adenovirus 5 DNA) that require Biosafety Level 2 according to the American Center for Disease Control and Prevention (CDC) guidelines. The biosafety level may vary depending on the country. For example, in Germany HEK293 cell lines are designated Biosafety Level 1 according to the Central Committee of Biological Safety, Zentrale Kommission für die Biologische Sicherheit (ZKBS). Please check with your country's regulatory authority regarding the use of these cells.

HANDLING PROCEDURES

Required Cell Culture Medium

- **Growth Medium:** DMEM, 4.5 g/l glucose, 2 mM L-glutamine, 10% heat-inactivated fetal bovine serum (FBS; 30 min at 56 °C), 100 µg/ml Normocin™, Pen-Strep (100 U/ml-100 µg/ml)
- **Freezing Medium:** DMEM, 4.5 g/l glucose, 20% FBS, 10% DMSO

Note: Some FBS may contain alkaline phosphatases that can interfere with SEAP quantification. We recommend to use heat-inactivated FBS to inactivate these thermosensitive enzymes.

- **Required Selection Antibiotics:** Puromycin and Zeocin™

Initial Culture Procedure

The first propagation of cells should be for generating stocks for future use. This ensures the stability and performance of the cells for subsequent experiments.

1. Thaw the vial by gentle agitation in a 37 °C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol.

Note: All of the steps from this point should be carried out under strict aseptic conditions.

3. Transfer cells to a larger tube containing 15 ml of pre-warmed growth medium. **Do not add selection antibiotics until the cells have been passaged twice.**
4. Centrifuge tube at 200-300 x *g* for 5 minutes.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium without selective antibiotics.
6. Transfer the contents to a T-25 tissue culture flask containing 5 ml of growth medium without selective antibiotics.
7. Place the culture at 37°C in 5% CO_2.

Frozen Stock Preparation

1. Resuspend cells at a density of 5-7x 10^6 cells/ml in freshly prepared freezing medium.

Note: A T-75 culture flask typically yields enough cells for preparing 3-4 frozen vials.

2. Dispense 1 ml of cell suspension into cryogenic vials.
3. Place vials in a freezing container and store at -80°C overnight.
4. Transfer vials to liquid nitrogen for long-term storage.

Note: If properly stored, cells should remain stable for years.

Cell maintenance

1. Maintain and subculture the cells in growth medium supplemented with 1 µg/ml of Puromycin and 100 µg/ml of Zeocin™.
2. Renew growth medium twice a week.
3. Cells should be passaged when a 70-80% confluency is reached. Do not let the cells grow to 100% confluency.

Note: The hACE2 surface expression may be altered by the action of trypsin. We recommend you add pre-warmed phosphate buffered saline (PBS) and detach cells by tapping the flask.

Cell Handling Recommendations

To ensure the best results, use HEK-Blue™ hACE2 cells with less than 20 passages.

CELL FUSION ASSAY

InvivoGen has developed a protocol for studying cell fusion using our engineered 293-hMyD88 and HEK-Blue™ hACE2 as 'donor' and 'acceptor' cells, respectively.

Note: For more information on the handling and preparation of the 293-hMyD88 cells please visit https://www.invivogen.com/hek-hmyd88

Generation of "donor cells" using 293-hMyD88 cells

1. Wash 293-hMyD88 cells with PBS and detach cells with trypsin.
2. Centrifuge cells at 300 x g (RCF) for 5 min.
3. Remove supernatant and resuspend cells at 0.3 x 10^6 cells/ml in fresh, pre-warmed growth medium
4. Add 3ml of cell suspension (~1.0 x 10^6 cells) per well of a 6-well plate.
5. Prepare LyoVec™ complex: Combine 1.5 µg pUNO1-SpikeV1 with 150 µL LyoVec™ and incubate at room temperature for 30 mins.

Note: InvivoGen offers a comprehensive collection of expression plasmids encoding various Spike variants (e.g. B.1.1.7, B.1.351, etc.). For more information: https://www.invivogen.com/sars2-spike-vectors

6. Add 150 µl of prepared complex to the cell-containing wells.
7. Incubate the plate for 24h or 48h at 37°C, 5% CO_2.

Preparation of HEK-Blue™ hACE2 cells

1. Gently rinse HEK-Blue™ hACE2 cells twice with pre-warmed PBS and detach the cells in PBS by tapping the flask. Dissociate cell clumps by gently pipetting up and down.

Note: Do not use trypsin to detach HEK-Blue™ hACE2 cells.

2. Centrifuge cells at 300 x g (RCF) for 5 min.
3. Remove supernatant and prepare a suspension at 2.0 x 10^5 cells/ml in fresh, pre-warmed growth medium.

Co-culture of 'donor' and 'acceptor' cells

1. Wash pre-prepared transfected cells (293-hMyD88-Spike) with PBS and detach in PBS by tapping the plate.
2. Centrifuge cells at 300 x g (RCF) for 5 min.
3. Remove supernatant and prepare a suspension at 1.0 x 10^6 cells/ml in fresh, pre-warmed growth medium.
4. Prepare a 1:2 serial dilution of the 293-hMyD88-Spike cells in a 96-well plate, starting with a final concentration of 1.0 x 10^5 cells/well. Final volume of 100 µl per well.
5. Add 100 µl of prepared HEK Blue™ hACE2 cell suspension per well (20,000 cells/well).
7. Incubate the plate for 24h at 37°C, 5% CO_2.

Measuring cell fusion

1. Prepare QUANTI-Blue™ Solution as per the product data sheet.
2. Dispense 180 µl of QUANTI-Blue™ Solution per well of a new flat-bottom 96-well plate.
3. Add 20 µl of cell fusion supernatant per well.
4. Incubate the plate at 37°C for 1-3 h.
5. Determine SEAP levels using a spectrophotometer at 620-655 nm.

RELATED PRODUCTS

Product	Cat. Code
Puromycin	ant-pr-1
Zeocin™	ant-zn-1
293-hMyD88 Cells	293-hmyd
pUNO1- SpikeV1	p1-spike-v1

TECHNICAL SUPPORT
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QUANTI-Blue™ Solution

Medium for detection and quantification of alkaline phosphatase in standard and HTS assays

Catalog code: rep-qbs, rep-qbs2, rep-qbs3

https://www.invivogen.com/quanti-blue

For research use only

Version 20C16-MM

PRODUCT INFORMATION

Contents: QUANTI-Blue™ Solution is available in three pack sizes

- **rep-qbs:** 5 x 1 ml of QB reagent and 5 x 1 ml QB buffer, sufficient to prepare QUANTI-Blue™ Solution for **25 x 96-well plates** (500 ml using the standard procedure) or **20 x 1536-well plates** (85 ml using the HTS screening procedure).
- **rep-qbs2:** 10 x 1 ml of QB reagent and 10 x 1 ml QB buffer, sufficient to prepare QUANTI-Blue™ Solution for **50 x 96-well plates** (1 L using the standard procedure) or **40 x 1536-well plates** (170 ml using the HTS screening procedure).
- **rep-qbs3:** 1 x 20 ml bottle of QB reagent and 1 x 20 ml bottle of QB buffer, sufficient to prepare QUANTI-Blue™ Solution for **100 x 96-well plates** (2 L using the standard procedure) or **80 x 1536-well plates** (340 ml using the HTS screening procedure).

Required Material (not provided)

- Sterile water
- Sterile screw cap tube, glass bottle or flask

Storage and stability

- Product is shipped at room temperature. Upon receipt, store QB reagent and QB buffer at -20 °C. Product is stable for 1 year at -20 °C when properly stored.
- The 20 ml bottles of QB reagent and QB buffer are designed for single use. If required, individual aliquots of QB reagent and QB buffer can be prepared upon receipt or following a single freeze-thaw cycle. Store aliquots at -20°C. **Avoid repeated freeze-thaw cycles**.

Note: During storage, a precipitate may form in the 20 ml bottle of QB reagent. If this occurs, vortex the product until the precipitate disappears. The formation of a precipitate does not affect the activity of the product.

- Reconstituted QUANTI-Blue™ Solution is stable for 2 weeks at 2-8 °C and for 2 months at -20 °C. Protect QUANTI-Blue™ from light.

Quality Control

Each lot is thoroughly tested to ensure the absence of lot-to-lot variation.

- Physicochemical characterization (including pH, solubility).
- Functional assays using alkaline phosphatase or SEAP-expressing reporter cells.

DESCRIPTION

QUANTI-Blue™ is a colorimetric enzyme assay developed to determine any alkaline phosphatase activity (AP) in a biological sample, such as supernatants of cell cultures. QUANTI-Blue™ Solution changes from pink to a purple-blue color in the presence of AP. Secreted embryonic alkaline phosphatase (SEAP) is a widely used reporter gene. It is a truncated form of placental alkaline phosphatase, a glycosylphosphatidylinositol (GPI)-anchored protein. SEAP is secreted into the cell culture supernatant and therefore offers many advantages over intracellular reporters.

QUANTI-Blue™ is highly sensitive for quantitative measurement. It has a higher saturation threshold than with pNPP (p-nitrophenyl phosphate) resulting in more significant differences between no, low or high AP activity. Another advantage of QUANTI-Blue™ is that it can determine secreted AP activity without disturbing cells, thus allowing the repeated sampling of cell cultures for kinetic studies.

METHODS

QUANTI-Blue™ Solution has been optimized for use in 96-well plates (standard procedure) and in 1536-well plates (high throughput screening procedure).

A. Standard procedure

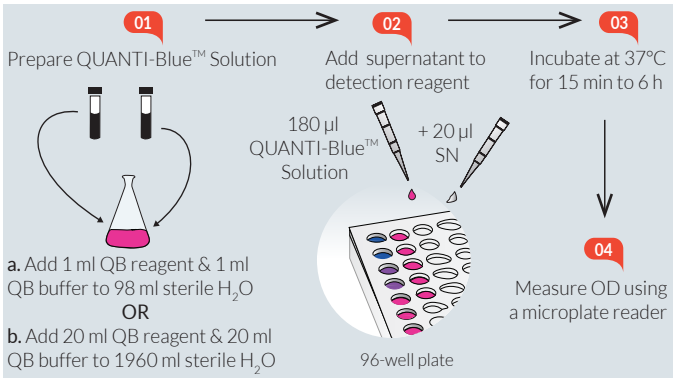

Figure 1. Standard procedure using QUANTI-Blue™ Solution.

The following protocol refers to the use of 96-well plates. Ensure QB reagent and QB buffer are completely thawed before use.

*Note: For fast thawing, QB reagent and QB buffer can be placed at 37 °C for 2 minutes. Ensure heating at 37 °C does **not** exceed 5 minutes.*

1. In a sterile bottle or flask, prepare QUANTI-Blue™ Solution by adding:
   a. **1 ml** of QB reagent and **1 ml** of QB buffer to **98 ml** of sterile water.
   b. **20 ml** of QB reagent and **20 ml** of QB buffer to **1960 ml** of sterile water.
2. Mix by vortexing and incubate at room temperature for 10 min before use.
3. Use QUANTI-Blue™ Solution immediately or store at 2-8 °C or -20 °C.
4. Dispense 180 µl of QUANTI-Blue™ Solution per well into a flat-bottom 96-well plate.
5. Add 20 µl of the sample (supernatant of SEAP-expressing cells) or negative control (cell culture medium).
6. Incubate at 37 °C for 15 min to 6 h.
7. Measure optical density (OD) at 620-655 nm using a microplate reader.

Note: If the negative control turns purple/blue, it means the fetal bovine serum (FBS) contains alkaline phosphatase. We recommend heating FBS at 56 °C for 30 min to inactivate the alkaline phosphatase activity.

For different cell culture plate formats, please refer to the table below:

	96-well plate	24-well plate	12-well plate
QUANTI-Blue™	180 µl	450 µl	900 µl
Supernatant	20 µl	50 µl	100 µl

TECHNICAL SUPPORT
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B. High Throughput Screening (HTS) procedure

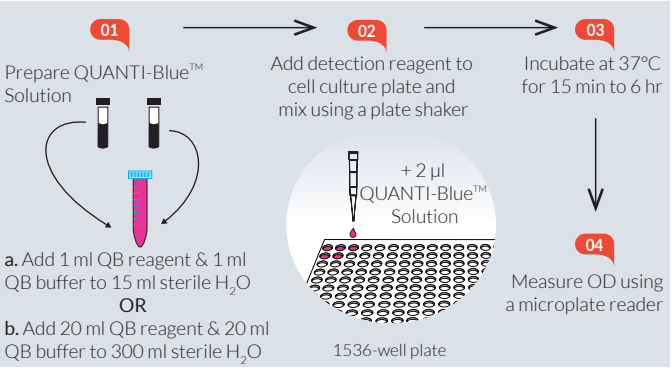

Figure 2. High throughput screening procedure using QUANTI-Blue™ Solution.

This procedure has been optimized for use in HTS screening procedures in 1536-well plates. QUANTI-Blue™ Solution is added directly to the cell suspension to reduce liquid handling.
Ensure QB reagent and QB buffer are completely thawed before use.
Note: For fast thawing, QB reagent and QB buffer can be placed at 37 °C for 2 minutes. Ensure heating at 37 °C does ***not*** *exceed 5 minutes.*

1. Dispense cell suspension and test compounds into a 1536-well plate in a volume that does not exceed **5 µl** per well. Incubate cells with test compounds for the desired period of time.
2. Prepare QUANTI-Blue™ Solution by adding:
   a. **1 ml** of QB reagent and **1 ml** of QB buffer to **15 ml** of sterile water in a sterile 50 ml screw cap tube.
   b. **20 ml** of QB reagent and **20 ml** of QB buffer to **300 ml** of sterile water in a sterile glass bottle or flask.
3. Mix well by vortexing and incubate at room temperature for 10 minutes before use.
4. Use QUANTI-Blue™ Solution immediately or store at 2-8 °C or -20 °C.
5. Dispense **2 µl of QUANTI-Blue™ Solution** to the wells containing ≤ 5 µl of cell culture in a 1536-well plate.
6. Mix using a plate shaker.
7. Incubate at 37 °C for 15 min to 6 h.
8. Measure OD at 620-655 nm.

Note: If the negative control turns purple/blue, it means the fetal bovine serum (FBS) contains alkaline phosphatase. We recommend heating FBS at 56 °C for 30 min to inactivate the alkaline phosphatase activity.

RELATED PRODUCTS

Product	Catalog Code
pNiFty2-SEAP (Zeo®)	pnifty2-seap
pSELECT-zeo-SEAP	psetz-seap
HEK-Blue™ Detection	hb-det2
Recombinant SEAP Protein	rec-hseap
Reporter cells	
HEK-Blue™ hTLR2	hkb-htlr2
HEK-Blue™ hTLR4	hkb-htlr4
RAW-Blue™ Cells	raw-sp
THP1-Blue™ NF-κB Cells	thp-nfkb
THP1-Blue™ ISG Cells	thp-isg

For a complete list of InvivoGen's Reporter Cell Lines visit
https://www.invivogen.com/reporter-cells